(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 June 2003 (26.06.2003)

PCT

(10) International Publication Number
WO 03/051403 A1

(51) International Patent Classification?: A61K 51/06, A61P 19/00

(21) International Application Number: PCT/US02/39692

(22) International Filing Date: 11 December 2002 (11.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/340,665 13 December 2001 (13.12.2001) US
60/340,545 13 December 2001 (13.12.2001) US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/051403 A1

(54) Title: TREATMENT OF OSTEOMYELITIS WITH RADIOPHARMACEUTICALS

(57) Abstract: This invention relates to medical uses of radiopharmaceuticals. Specifically, the present invention relates to the use of radiopharmaceuticals to treat osteomyelitis. The present invention provides improved system and methods of for the direct delivery of radiopharmaceuticals to the site of osteomyelitis.

As used herein, the term "pharmaceutical composition comprising a radionuclide" refers to any pharmaceutically acceptable composition that comprises a radionuclide and any sterile, biocompatible pharmaceutical carrier. Pharmaceutical compounds may also include additional active agents such as, including but not limited to, ligands complexed to the radionuclides.

The terms "pharmaceutically acceptable" and "pharmacologically acceptable," as used herein, refer to compositions that do not substantially produce adverse allergic, immunological or other reactions when administered to a host (for example, an animal such as a human). As used herein, "pharmaceutically acceptable carrier" includes any and all solvents (for example, including but not limited to, saline, buffered saline, dextrose and water), dispersion media, coatings, wetting agents (for example, sodium lauryl sulfate), isotonic and absorption delaying agents, and disintrigrants (for example, potato starch or sodium starch glycolate).

As used herein, the term "locally administering a composition comprising a radionuclide to subject suffering from osteomyelitis at said site of infection" refers to administering a pharmaceutical composition of the present invention directly to the site of osteomyelitis (for example, a limb bone).

As used herein, the term "ligand" refers to any compound capable of physically interacting with a radionuclide of the present invention. In some embodiments, the radionuclide is chelated by electron donor groups of the ligand. However, any interaction that results in stable complexes when administered to a subject using the methods of the present invention is suitable. The term "ligand" is also not intended to be limited by the chemical nature of the compound. In preferred embodiments, a macrocyclic or acyclic aminophosphonic acid is used as a ligand for complexing with a radionuclide of the present invention.

The term "cyclic compounds" refers to compounds having one (that is, a monocyclic compounds) or more than one (that is, polycyclic compounds) ring of atoms. The term is not limited to compounds with rings containing a particular number of atoms. While most cyclic compounds contain rings with five or six atoms, rings with other numbers of atoms (for example, three, four, or twelve atoms) are also contemplated by the present invention. The identity of the atoms in the rings is not limited, though the atoms are usually predominantly carbon atoms. Generally speaking, the rings of polycyclic compounds are

adjacent to one another. However, the term "polycyclic compound" includes those compounds containing multiple rings that are not adjacent to each other.

The terms "macrocyclic compound" and "macrocycle" refer to a "cyclic compound" with a ring containing more than about eight atoms.

The term "heterocyclic compounds" refers broadly to cyclic compounds wherein one or more of the rings contains more than one type of atom. In general, carbon represents the predominant atom, while the other atoms include, for example, nitrogen, sulfur, and oxygen. Examples of heterocyclic compounds include benzimidazole, furan, pyrrole, thiophene, and pyridine.

As used herein, the term "parenteral administration" includes all routes of administering an agent (for example, a pharmaceutical composition of the present invention) that are not through the gastrointestinal route. Examples of parenteral administration include, but are not limited to, intravenous, intra-arterial, intramuscular, local, subcutaneous, intradermal, and transcutaneous administration.

The terms "aromatic" and "aromatic compounds" refer broadly to compounds with rings of atoms having delocalized electrons. The monocyclic compound benzene (C_6H_6) is a common aromatic compound. However, electron delocalization can occur over more than one adjacent ring (for example, naphthalene [two rings] and anthracene [three rings]). Different classes of aromatic compounds include, but are not limited to, aromatic halides (aryl halides), aromatic heterocyclic compounds, aromatic hydrocarbons (arenes), and aromatic nitro compounds (aryl nitro compounds).

As used herein the terms "meta substitution" and "meta position" when used in terms of substituted benzenes, refer to benzene derivatives substituted at positions 1 and 3 or 1 and 5 (that is, each of the 6 carbons of the 6 membered benzene ring is numbered consecutively). As used herein, the terms "para substitution" and "para position" when used in terms of substituted benzenes, refer to benzene derivatives substituted at positions 1 and 4 of the benzene ring.

As used herein, the terms "aliphatic" and "aliphatic compounds" refer to compounds which comprise carbon atoms in chains, rather than the ring structure of cyclic compounds.

The term "mixture" refers to a mingling together of two or more substances without the occurrence of a reaction by which they would lose their individual properties. The term "solution" refers to a liquid mixture. The term "aqueous solution" refers to a solution that contains some water. In many instances, water serves as the diluent for solid substances to

create a solution containing those substances. In other instances, solid substances are merely carried in the aqueous solution (that is, they are not dissolved therein and are a "mixture" of an aqueous solution and the non-dissolved solid substances). The term aqueous solution also refers to the combination of one or more other liquid substances with water to form a multi-component solution.

"Acylate" as used herein, refers to the introduction of an acyl group into a molecule, (that is, acylation).

"Biologically active", as used herein, refers to a molecule having the structural, regulatory, or biochemical functions of a naturally occurring molecule.

"Cell culture" as used herein, refers to a proliferating mass of cells that may be in either an undifferentiated or differentiated state.

"Immunologically active" refers to the capability of a natural, recombinant, or synthetic polypeptide, or any oligopeptide thereof, to bind with specific antibodies and induce a specific immune response in appropriate animals or cells.

"Purified" as used herein when referring to a chemical compound or molecule, indicates that the molecule is present in the substantial absence of other chemical or biological compounds of the same type. The term "purified" as used herein preferably means at least 95 percent by weight, more preferably at least 99.8 percent by weight, of molecules of the same type present.

The term "pure" as used herein preferably has the same numerical limits as "purified" immediately above.

"Sample" as used herein, is used in its broadest sense. A biological sample may comprise a tissue, a cell, an extract from cells, blood, serum, and other bodily fluids.

The present invention provides methods for the treatment of osteomyelitis. Osteomyelitis is often diagnosed by a nuclear medicine bone scan using known radiopharmaceutical agents. The radiopharmaceuticals concentrate at the site of bone infection to show the presence of infection. Radiopharmaceuticals have also been used to treat bone cancers, arthritis, and to ablate bone marrow.

Accordingly, the present invention provides methods for the use of radiopharmaceuticals to treat osteomyelitis. The methods of the present invention find use in the treatment of all forms of osteomyelitis (for example, acute or chronic infection). The present invention further provides delivery methods that increase the localization of the radioactivity to the bone, thus reducing the systemic radiation dose.

The methods and compositions described below are exemplary and are not intended to limit the scope of the invention. One skilled in the relevant art recognizes that additional suitable radiopharmaceuticals, ligands, dosages, and treatment formulations may be substituted for those disclosed herein.

I. Radiopharmaceuticals

The present invention provides radiopharmaceuticals for the treatment of osteomyelitis. The invention is not limited to a particular radioisotope or ligand. Any suitable radioisotope or ligand that functions to treat osteomyelitis may be utilized, including but not limited to, those disclosed herein. Guidance for selecting and screening agents for use in the methods of the present invention are described below.

A. Radionuclides

The radiopharmaceutical compositions of the present invention comprise one or more radionuclides. In preferred embodiments, the half-life of the radionuclides is sufficiently long to allow for localization and delivery of the complex in the bone tissue while still retaining sufficient radioactivity to kill pathogens present in the bone. Generally, it is preferred to use a radionuclide-ligand complex that results in rapid biolocalization of the radionuclide in the bone tissue so as to achieve rapid onset of pathogen irradiation. In preferred embodiments, a radionuclide having sufficient alpha or beta energy is utilized.

By preferentially delivering the radionuclide to the site of active bone infection, the radiation can be performed with nuclides that emit radiation with relatively short path lengths before absorption (for example, beta radiation) with good microbe kill and less damage to other tissues. In addition, directly targeting the radionuclide to the site of infections allows the use of a nuclide with a relatively short half life (for example, one or two days) that delivers its radiation dose quickly. This results in a higher likelihood that more of the pathogen will be killed. This is in direct contrast to the currently available methods of delivering a lower per minute dose of radiation over a longer time period that has the potential to allow more bacteria to repair any radiation damage and survive the treatment.

For example, in some embodiments, radionuclides utilized in the methods of the present invention exhibit beta energy >0.5 MeV, preferably >1 MeV with an effective half-life of <5 days, preferably < 3 days. Radionuclides useful in the methods and compositions of the present invention include, but are not limited to, Arsenic-77 (^{77}As), Molybdenum-99 (^{99}Mo), Rhodium-105 (^{105}Rh), Lutetium-177 (^{177}Lu), Cadmium-115 (^{115}Cd), Antimony-122

(^{122}Sb), Promethium-149 (^{149}Pr), Osmium-193 (^{193}Os), Gold-198 (^{198}Au), Tin-117m (^{117m}Sn), Strontium-89 (^{89}Sr), Thorium-200 (^{200}Th) Indium-115 (^{115}In), Dysprosium-165 (^{165}Dy), Lanthanum-140 (^{140}La), Ytterbium-175 (^{175}Yb), Scandium-47 (^{47}Sc); preferably Samarium-153 (^{153}Sm), Yttrium-90 (^{90}Y), Gadolinium-159 (^{159}Gd), Rhenium-186 (^{186}Re), Rhenium-188 (^{188}Re), and Holmium-166 (^{166}Ho). Especially preferred is ^{166}Ho, which emits high-energy beta particles and gamma radiation (80 KeV, 6.0 percent) useful for imaging and exhibits a half-life of 26.8 hr. In other embodiments, alpha emitters such as Actinium-225 (^{225}Ac), Bismuth-212 (^{212}Bi) and Bismuth-213 (^{213}Bi) are utilized.

The respective radionuclides can be obtained using procedures well known in the art. Typically, the desired radionuclide can be prepared by bombarding an appropriate target, such as a metal, metal oxide, or salt with neutrons. Another method of obtaining radionuclides is by bombarding nuclides with particles in a linear accelerator or cyclotron. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures. The present invention is not limited to a particular method of obtaining radionuclides. Any suitable method that results in the generation of the desired radionuclide may be utilized.

B. Ligands

In some embodiments of the present invention, radionuclides are conjugated to pharmaceutically acceptable ligands. In particularly preferred embodiments, aminophosphonic acids, particularly macrocyclic and acyclic aminophosphonic acids, are utilized as ligands. These compounds are prepared by any suitable technique. Known synthetic techniques involve reacting a compound containing at least one reactive amine hydrogen with a carbonyl compound (aldehyde or ketone) and a phosphorous acid or appropriate derivative thereof.

Methods for carboxyalkylating macrocyclic amines to give amine derivatives containing a carboxylalkyl group are disclosed in U.S. Patent 3,726,912. Methods to prepare alkylphosphonic acid amines and hydroxyalkylamines are disclosed in U.S. Patents 3,398,198, 5,066,478, and 5,300,279.

The amine precursor (1,4,7,10-tetraazacyclododecane) employed in making certain of the macrocyclic aminophosphonic acids utilized in some embodiments of the present invention is a commercially available material. The preparation of macrocyclic aminophosphonic ligands can also be found in U.S. Patent 5,059,412. The preparation of these ligands has also been described in U.S. Patents 4,973,333, 4,882,142, 4,853,209, 4,898,724, 4,897,254, 5,587,451, 5,714,604, 5,064,633, 5,587,451, 5,066,478, 5,300,279,

5,059,412, and 5,064,633. In preferred embodiments, ligands are selected from the group consisting of ethylenediaminetetramethylenephosphonic acid (EDTMP), diethylenetriaminepentamethylenephosphonic acid (DTPMP), hydroxyethylethylenediaminetrimethylenephosphonic acid (HEEDTMP), nitrilo-trimethylenephosphonic acid (NTMP), 1,4,7,10-tetraazacyclododecanetetra-methylenephosphonic acid (DOTMP), tris(2-aminoethyl)amine hexamethylene-phosphonic acid (TTHMP), methylene diphosphonate, hydroxymethylenediphosphonate, hydroxyethylidene diphosphonate (HEDP), and ethane-1-hydroxy-1,1-diphosphonic acid. In particularly preferred embodiments, ligands are macrocyclic aminophosphonic acid ligands of which 1,4,7,10-tetraazacyclododecanetetramethylenephosphonic acid (DOTMP) is an example.

In addition to phosphorus based chelates, aminocarboxylic acids such as diethylenetriaminepentaacetic acid can also be used to deliver isotopes to bone tissue. For example, U.S. Patent 6,231,832 teaches the delivery of Sn-117m to bone using such a chelator. Also, U.S. Patent 4,897,254 teaches the uses of hydroxyethylethylenediaminetriacetic acid in combination with Sm-153 to deliver a radiation dose to bone.

C. Radionuclide-Ligand Complexes

In preferred embodiments, the methods and compositions of the present invention employ complexes of radionuclides and ligands. The complexes may be generated using any suitable method, including but not limited to, those disclosed herein. In preferred embodiments, the radionuclide complex must be taken up preferentially by bone so that it is possible to deliver radiation to the bone with minimal exposure to other tissues such as lung, liver, bladder or kidneys. It is also preferred that the radionuclide complex be rapidly cleared from the blood, thereby further reducing exposure to non-target tissues.

The radionuclide and ligand are combined under any conditions that allow the two to form a complex. Generally, mixing in water at a controlled pH (the choice of pH is dependent upon the choice of ligand and radionuclide) is suitable. The complex is formed by chelation of the radionuclide by an electron donor group or groups that results in a stable radionuclide complex (for example, stable to the disassociation of the radionuclide from the ligand). For example, ^{166}Ho-DOTMP is formed by adding a ^{166}Ho salt, such as the chloride or nitrate in aqueous HCl (0.1 - 1 N), to a sterile, evacuated vial containing at least 3 equivalents of DOTMP in aqueous base (KOH and NaOH). After stirring at a pH of 10.5,

the pH is then adjusted to 7-8 by adding phosphate buffer and a stabilizing agent such as ascorbic acid. Complexation of >99 percent is generally achieved using such a method.

For the purpose of the present invention, radionuclide compositions described herein and physiologically acceptable salts thereof are considered equivalent. Physiologically acceptable salts refer to the acid addition salts of those bases which will form a salt with at least one acid group of the ligand or ligands employed and which will not cause adverse physiological effects when administered as described herein. Suitable bases include, but are not limited to, for example, the alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as, for example, sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate, amine hydroxides, carbonates, and bicarbonates such as, for example, ammonium hyroxide, ammonium carbonate, or primary secondary and tertiary amine hydroxides, carbonates, and bicarbonates such as, for example, trimethyl ammonium carbonate. Physiologically acceptable salts can be prepared by treating the macrocyclic aminophosphonic acid with an appropriate base.

The macrocyclic aminophosphonic acid complexes when formed at approximately a ligand to metal molar ratio of 1:1 to 20:1 give biodistributions that are consistent with those exhibited by known agents that are bone-specific. The optimum ratio depends on the particular ligand utilized. Preferred osteomyelitic treating radionuclide compositions include ^{166}Ho- DOTMP, ^{177}Lu-DOTMP, and ^{153}Sm-EDTMP. Preferably, molar ratios of DOTMP to ^{166}Ho are above 1, for example, from 1.5 to 3.5:1. The most preferred ratio is about 3.5:1. Such a ratio provides adequate complexation of the radionuclide while compensating for radiolysis of the ligand. By contrast, other acyclic aminophosphonic acid complexes can result in substantial localization of radioactivity in soft tissue (for example, liver) if large excess amounts of ligand are not used. Large excesses of ligand are undesirable since uncomplexed ligand may be toxic to the patient or may result in cardiac arrest or hypocalcemic convulsions. In addition, the macrocyclic aminophosphonic acid ligands are useful when large amounts of metal are required (that is, for metals that have a low specific activity). In this case, the macrocyclic aminophosphonic acid ligands have the ability to deposit more tolerable doses of radioactivity in the bone than is possible when using non-cyclic aminophosphonic acid ligands.

In the case of other ligands, such as EDTMP, a large excess of ligand is necessary. The most preferred ratio of EDTMP to Sm is 273:1. Aminocarboxylic acid ligands are also preferably present in large excess over radionuclide.

D. Pharmaceutical compositions

In preferred embodiments, radionuclides and radionuclide-ligand complexes are administered as pharmaceutically acceptable compositions. A pharmaceutically acceptable means of protecting the radionuclide complex from radiolytic decay of the chelator is highly preferred. Preferred radioprotectants of the present invention are radio-stable anti-oxidants, compounds that either reduce the number or the activity of oxidizing radicals. Exemplary radio protectants that can be employed in the practice of the present invention are ascorbic acid, gentisic acid, nicotinic acid, ascorbyl palmitate, HOP(:O) H_2, monothioglycerol, sodium formaldehyde sulfoxylate, $Na_2S_2O_3$, SO_2, or a reducing agent combined with BHA, BHT, pyrogallate, and tocopherol. Ascorbic acid is the preferred radioprotectant for use in the practice of the present invention, and can be used at 35-75 mg/ml of liquid composition. This concentration of ascorbate can provide a solution of ^{166}Ho-DOTMP that is stable (for example, therapeutically useful), for at least 72 hours at ambient conditions (for example, unfrozen).

The formulations of the present invention are in the solid or preferably liquid form containing the active radionuclide complexed with the ligand. These formulations can be in kit form such that the chelator and radionuclide are mixed at the appropriate time prior to use in a suitable liquid carrier with the radioprotectant. Whether premixed or as a kit, the formulations usually require a pharmaceutically acceptable carrier, such as water.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile solutions, dispersions, emulsions, or microemulsions, comprising the active ingredients that are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in protective matrices such as nanoparticles or microparticles. In all cases, the ultimate dosage form must be sterile, fluid, and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycols), DMSO, and suitable mixtures thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride.

Injectable suspensions as compositions of the present invention require a liquid suspending medium, with or without adjuvants, as a carrier. The suspending medium can be, for example, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous carboxymethyl cellulose solutions. If necessary to keep the complex in suspension, suitable physiologically acceptable adjuvants can be chosen from among thickeners such as, for example, carboxymethylcellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many surfactants are also useful as suspending agents, for example, lecithin, alkylphenol, polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylenesorbitan esters. Many substances that effect the hydrophobicity, density, and surface tension of the liquid suspension medium can assist in making injectable suspensions in individual cases. For example, silicone antifoams, sorbitol, and sugars are all useful suspending agents.

II. Treatment of Osteomyelitis

The present invention provides novel methods of treating osteomyelitis using radiopharmaceutical compositions. In some preferred embodiments, the compositions are delivered directly to the site of infection, thus decreasing the amount of radioactivity required to reduce infection. The present invention is not limited to the dosages and methods of administration described below. One skilled in the art recognizes that other suitable dosages and administration methods may be utilized in the practice of the present invention.

A. Dosages

The effective therapeutic amount of radionuclide composition administered to achieve elimination of osteomyelitis will vary according to factors such as the age, weight and health of the patient, the disease state being treated (for example, chronic or acute infection), the treatment regimen selected (for example, mode of administration), the amount of oxygen in the system, as well as the nature of the particular radionuclide composition to be administered. For example, less activity will be needed for radionuclides with longer half lives. The energy of the emissions will also be a factor in determining the amount of activity necessary. In some embodiments, a dose of 10 to 1000 Gy is used. Preferably, a total dose of 20-60 Gy, most preferably 30-60 Gy, for example, 40-50 Gy of radiation is delivered to bone parenterally (for example, preferably via intramuscular injection or locally).

The radiation exposure is reported using the Grey scale (Gy). One Gy is equivalent to 100 Rads. A rad is defined as adsorbed energy of 100 ergs per gram. Because the biodistribution of radiopharmaceuticals vary from patient to patient, it is preferred to first administer a small dose and determine the biodistribution of the agent prior to giving the therapeutic dose. Radioactivity measurements of the isotope in blood, urine, bone, and infected areas are used to estimate the dose to the target and non-target areas. This is translated into a therapeutic dose for the individual patient. For example, in some embodiments, a diagnostic dose of 1110-1850 MBq (30mCi to 50 mCi) of Ho-166-DOTMP is used as a diagnostic dose to determine the therapeutic dose. Alternatively, a different agent, such as Tc-99m-MDP, that has a very similar biodistribution as the therapeutic agent can be given prior to the therapeutic dose. Determination of the doses of radiation delivered by the present complexes can be determined in accord with known methodologies (See for example, Bardies et al., Physics in Medicine and Biology, 41,1941 (1996); Beddoe et al., Physics in Medicine & Biology, 21, 589 (1976); Bigler et al., Health Physics, 31, 213 (1976); Champlin et al., Semin. Hematol, 24, 55 (1987); Champlin et al., Cancer Treatment Reports, 68, 145 (1984); Eckerman et al., Journal of Nuclear Medicine, 35, 112P (1994); Spiers et al., British Journal of Radiology, 54, 500 (1981)).

B. Additional Therapeutic Agents

In some embodiments, radiopharmaceuticals are administered in combination with additional agents (for example, including but not limited to, antibacterial, anti-parasitic, and antifungal agents, including those disclosed in The Physicians Desk Reference, 50th Edition, 1996).

Useful antibiotic agents include systemic antibiotics, such as aminoglycosides, cephalosporins (for example, first, second, and third generation), macrolides (for example, erythromycins), monobactams, penicillins, quinolones, sulfonamides, and tetracyclines, including those disclosed in The Physicians Desk Reference, 50th Edition, 1996. In addition, antibacterial agents include 2-isocephem and oxacephem derivatives disclosed in U.S. Patent 5,919,925; pyridone carboxylic acid derivatives disclosed in U.S. Patent 5,910,498; water miscible esters of mono- and diglycerides disclosed in U.S. Patent 5,908,862; benzamide derivatives disclosed in U.S. Patent 5,891,890; 3-ammoniopropenyl cephalosporin compounds disclosed in U.S. Patent 5,872,249; 6-O- substituted ketolides disclosed in U.S. Patent 5,866,549; benzopyran phenol derivatives disclosed in U.S. Patent 5,861,430; pyridine derivatives disclosed in U.S. Patent 5,859,032; 2-aminothiazole

derivatives disclosed in U.S. Patent 5,856,347; penem ester derivatives disclosed in U.S. Patent 5,830,889; lipodepsipeptides disclosed in U.S. Patent 5,830,855; dibenzimidazole derivatives disclosed in U.S. Patent 5,824,698; alkylenediamine derivatives disclosed in U.S. Patent 5,814,634; organic solvent-soluble mucopolysaccharides disclosed in U.S. Patent 5,783,570; arylhydrazone derivatives disclosed in U.S. Patent 5,760,063; carbapenem compounds disclosed in U.S. Patent 5,756,725; N-acylpiperazine derivatives disclosed in U.S. Patent 5,756,505; peptides disclosed in U.S. Patent 5,714,467; oxathiazines and their oxides disclosed in U.S. Patent 5,712,275; 5-amidomethyl alpha beta-saturated and -unsaturated 3-aryl butyolactone compounds disclosed in U.S. Patent 5,708,169; halogenated benzene derivatives disclosed in U.S. Patent 5,919,438; sulfur-containing heterocyclic compounds disclosed in U.S. Patent 5,888,526; and oral antibacterial agents disclosed in U.S. Patent 5,707,610.

Antifungal agents include dermatological fungicides, topical fungicides, systemic fungicides, and vaginal fungicides, including those disclosed in The Physicians Desk Reference, 50th Edition, 1996. In addition, antifungal agents include terpenes, sesquiterpenes, diterpenes, and triterpenes disclosed in U.S. Patent 5,917,084; sulfur-containing heterocyclic compounds disclosed in U.S. Patent 5,888,526; carbozamides disclosed in U.S. Patent 5,888,941; phyllosilicates disclosed in U.S. Patent 5,876,738; corynrcandin derivatives disclosed in U.S. Patent 5,863,773; sordaridin derivatives disclosed in U.S. Patent 5,854,280; cyclohexapeptides disclosed in U.S. Patent 5,854,213; terpene compounds disclosed in U.S. Patent 5,849,956; agents derived from aspergillus fumigatus disclosed in U.S. Patent 5,873,726; inula extracts disclosed in U.S. Patent 5,837,253; lipodepsipeptides disclosed in U.S. Patent No. 5,830,855; polypeptides disclosed in U.S. Patent 5,824,874; pyrimidone derivatives disclosed in U.S. Patent 5,807,854; agents from sporormiella minimizes disclosed in U.S. Patent 5,801,172; cyclic peptides disclosed in U.S. Patent 5,786,325; polypeptides disclosed in U.S. Patent 5,773,696; triazoles disclosed in U.S. Patent 5,773,443; fusacandins disclosed in U.S. Patent 5,773,421; terbenzimidazoles disclosed in U.S. Patent 5,770,617; and agents obtained from hormones disclosed in U.S. Patent 5,756,472.

C. Delivery of Radiopharmaceuticals

The present invention contemplates the use of radiopharmaceuticals to treat osteomyelitis in animals, including but not limited to, humans. The methods of the present invention are suitable to treat acute or chronic osteomyelitis in any bone. Suitable

radiopharmaceuticals, ligands, and dosages include, but are not limited to, those described above. One skilled in the relevant art understands how to determine suitable compositions and dosages for a specific animal and site or extent of infection.

The direct administration methods of the present invention provide the advantage of delivering an increased concentration of the radionuclide to the affected area and decreasing the exposure of the rest of the body. This is in contrast to systemic intravenous injection of bone agents, which results in radioactivity deposited in the entire skeletal system of the subject. The dose from the bone to the bone marrow is of most concern. This is especially true of radionuclides such as Ho-166 or Y-90 that are high-energy beta emitters. If a portion of the marrow can be spared from radioactivity, then it is probable that the affected area will regenerate without putting the patient at risk. Application to the infected area after isolating the blood flow using an arterial obstruction device, such as a tourniquet, will result in a larger dose to the infected area and reduce the dose to bone marrow. Similarly, application of one or more arterial obstruction devices to isolate portions of the skeletal system from the site of injection of the radionuclide will protect those portions of the bone marrow.

Accordingly, in preferred embodiments, radiopharmaceuticals are administered locally to the area of the infected bone. Local administration can be performed by techniques known in the art, including but are not limited to, intravenous injection, intra-arterial injection, intramuscular injection, subcutaneous injection, intraosseous injection, and transcutaneous administration. In some embodiments, radiopharmaceuticals are injected intramuscularly near the site of infection.

In some preferred embodiments, a tourniquet or other arterial obstruction device is placed above the area of injection in order to aid in the localization of the radiopharmaceuticals. In some embodiments, the tourniquet is placed on the limb prior to injection and removed immediately following injection. In preferred embodiments, the tourniquet is left in place for a short period of time following injection (for example, long enough for the radiopharmaceutical to localize to the site of infection). In preferred embodiments, the tourniquet is left in place for greater than 2 minutes (for example, preferably 5 minutes and more preferably 10 minutes) and then removed. It is preferred that the tourniquet is left in place no greater than 60 minutes in order to avoid hypoxic damage to the tissues due to restricted blood flow.

In other embodiments (for example, where the osteomyelitis is located in an area proximal to the most proximal limb location for a tourniquet, such as in a rib, vertebrae, pelvis, femoral head, humeral head, clavicle, scapula, skull, or mandible), tourniquets are applied to one or more extremities to prevent access of the radiopharmaceutical agent to these areas. For instance, in some embodiments, tourniquets are applied proximally to each leg to protect the bone marrow in the legs from exposure to the radiopharmaceutical. The radiopharmaceutical agent is then given intravenously into a brachial vein to be carried through the blood stream to an osteomyelitis site, for instance in the mandible or the vertebral column. The tourniquet is then removed after an appropriate time (less than 60 minutes but greater than 2 minutes, more preferably greater than 5 minutes, most preferably 10 minutes) after injection.

In some embodiments, radiopharmaceuticals are administered with additional antibacterial or fungal agents. Suitable agents include, but are not limited to, those described above.

In preferred embodiments, administration of a radiopharmaceutical agent result in the reduction of osteomyelitis (for example, as determined by a bone scan). If the infection is not sufficiently reduced or eliminated, additional doses of radiopharmaceuticals are given. Alternatively, or in combination, increased doses of radiopharmaceuticals are administered until symptoms and diagnostic tests reveal that the infection is eliminated.

EXAMPLES

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); °C (degrees Centigrade); U (units), mU (milliunits); Ci (Curie); min. (minutes); and sec. (seconds).

Example 1

Preparation of ^{166}Ho-DOTMP

165-Ho-nitrate targets were prepared from dissolution of holmium oxide in nitric acid followed by reduction to dryness. A target containing 6 mg of holmium was irradiated

in a reactor for approximately 155 hours at a flux of 4.5×10^{14} n/cm^2/s. The specific activity was typically in the range of 1.3 - 2 Ci/mg.

The ^{166}Ho-nitrate target was dissolved in 0.3 N HC1. In a typical 9 Ci preparation, ^{166}Ho-chloride was supplied from the reactor in 10 ml of 0.3 N HC1. DOTMP (60 mg DOTMP and 168 mg NaOH) was dissolved in 4 ml water and added to the ^{166}Ho chloride. The ligand to metal ratio was 3.5. The reaction mixture was allowed to mix for 10 minutes at a pH of 10.5. This was followed by addition of 4.8 ml of 1.0 M sodium phosphate buffer and ascorbic acid. The final concentration of ascorbic acid was 55 mg/ml. Dilution with water was performed to assure that the final activity concentration did not exceed 322 mCi/ml. The pH of the final product was 7 - 8.

Example 2

Treatment of Osteomyelitis in Rats with ^{166}Ho-DOTMP

This example describes the successful treatment of osteomyelitis in rats using ^{166}Ho-DOTMP. Four 150 g male Sprague Dawley rats were anesthetized and prepared for surgery by shaving the left leg. The skin over the left tibia and fibula was opened, a hole was drilled into the bone marrow, and an 18-gauge needle was inserted into the bone marrow. Through the needle, a piece of 0 surgical suture and approximately 0.1 ml of Staphylococcus aureus culture in Trypticase soy broth were introduced into the bone marrow. The needle was removed and the bone defect sealed with cyanoacrylate glue. The skin was closed with 0 surgical suture. The rats were followed with serial radiographs of the left leg. Lytic lesions diagnostic of osteomyelitis developed in the fibula over the next three weeks. The entire bone appeared radiolucent.

Two rats were followed without treatment. One week later, both of the rats died. Necropsy revealed that the tibia was eroded with only a very thin layer of bone encasing a thick fluid. Culture of the fluid grew Gram positive cocci consistent with the original Staphylococcus aureus infection.

Two rats were treated with ^{166}Ho-DOTMP. A tourniquet was placed on the left leg above the knee. An intramuscular injection of 30 Gray (9 milliCurie) of ^{166}Ho-DOTMP was given after application of the tourniquet. The tourniquet was released after 10 minutes. The rats were followed with continued serial radiographs of the leg. The radiographs showed a return to normal appearance of the tibia and fibula in two weeks.

Examples 3 through 6

Biodistribution of radiopharmaceuticals in rats with and without the application of a tourniquet to a limb bone.

A. Methods

1. Preparation of DOTMP and EDTMP

DOTMP and EDTMP were prepared according to methods described in U.S. Patent Nos. 4,898,724 and 4,976,950.

2. Preparation of Holmium and Samarium radionuclide solutions

Ho-166, obtained from the University of Missouri Research Reactor, Columbia Missouri, was dissolved in 0.1N HCl to yield a 6 x 10^{-3} M $^{166}HoCl_3$ solution. Sm-153, obtained from the University of Missouri Research Reactor, Columbia, Missouri, was dissolved in 0.1 N HCl to yield a 6.6 x 10^{-3} M solution.

The radioactive $^{166}HoCl3$ solution was then mixed with non-radioactive $^{165}HoCl3$ solutions to prepare solutions that would have only a tracer amount of ^{166}Ho. For complexation with DOTMP, 0.25 µL of the 6 x 10^{-3} M $^{166}HoCl_3$ solution was mixed with 1 mL of a 6.04 x 10^{-4} M $^{165}HoCl3$ solution. For complexation with EDTMP, 0.25 µL of the 6 x 10^{-3} M $^{166}HoCl_3$ solution was mixed with 1 mL of a 4.84 x 10^{-3} M $^{165}HoCl_3$ solution. These concentrations are chosen to fulfill the requirements of the DOTMP and EDTMP kits used for complexation.

Similarly, the radioactive $^{153}SmCl_3$ solution was mixed with non-radioactive $SmCl_3$ solutions to prepare solutions that would have only a tracer amount of ^{153}Sm. For complexation with DOTMP, 0.25 µL of the 6 x 10^{-3} M $^{153}SmCl_3$ solution was mixed with 1 mL of a 6.06 x 10^{-4} M solution of non-radioactive Sm $(CH_3COO)_3$. For complexation with EDTMP, 0.25 µL of the 6 x 10^{-3} M $^{153}SmCl_3$ solution was mixed with 1 mL of a 4.84 x 10^{-3} M solution of non-radioactive Sm $(CH_3COO)_3$.

3. Preparation and analysis of complexes

Preparation of EDTMP complexes and DOTMP complexes was accomplished by the methods described in U.S. Patent Nos. 4,898,724 and 4,976,950. Following preparation of the complexes, the percentage of complexation was determined. This was accomplished by placing an aliquot of the complexation solution onto a column of swollen Sephadex C-15 cation resin and eluting with a 4:1 physiologic saline:concentrated ammonium hydroxide solution. Percentage complexation can then be determined by comparison of the counts

from non-complexed metal left on the column to the total of counts from the non-complexed metal on the column and the complexed metal in the eluted solution.

4. Biodistribution studies

Rat biodistribution studies were done on Male Sprague-Dawley rats weighing 180-200g that had been acclimated for approximately one week prior to this study. Four test complexes were used, Ho-DOTMP (Example 3), Ho-EDTMP (Example 4), Sm-DOTMP (Example 5) and Sm-EDTMP (Example 6). The rats were placed in a restraining cage that afforded accessibility to the left hind leg and tail. Prior to injection a tie wrap tourniquet was placed on the left hind femur above the knee to restrict blood flow. The amount of pressure induced by the tourniquet was standardized by using a tie wrap gun and was sufficient to stop arterial flow. Three rats per complex each received a 100.0 µL intravenous injection in a lateral tail vein. The tourniquets were allowed to remain in place for 5, 10 or 20 minutes post injection and were then removed. The rats were then watched for two hours, the usual biodistribution period used to allow excretion of all radioactive complexes not bound to tissue. The rats were then sacrificed for removal of tibias. The number of radioactive counts were then determined and compared to the average of number of counts of three 100.0 uL standards of the same material that was injected. Several additional tissues (femur, injection site, liver, kidney, spleen, muscle, and blood) were collected and counted for rats that had the 20 minutes restricted blood flow to check for expected renal excretion of radioactive complexes.

B. RESULTS

Of the tissues checked, only the bone had significant radioactive counts present. This indicates normal clearance of the complexes not bound to bone and stability of the metal complexes.

Tables 1 and 2 show data as percent dose of administered radioactivity per gram of tissue. This reflects the concentration of the radioactive complex in the bone. Table 1 combines the data on the radioactivity found in the tibias of the rats from Examples 3 to 6 for each of the three times of tourniquet placement. Table 2 combines the data on the radioactivity found in the femurs of the rats from Examples 3 to 6 for the tourniquets applied for 20 minutes. The degree of "protection" of the bone by the tourniquet can be seen by comparison of the radioactivity in the bones on the left side ("protected" by the tourniquet for the time specified) as compared to the corresponding bones on the right side (fully exposed to the systemic, intravenous dose). Table 3 shows this comparison as the

percentage of the concentration of radioactive complex in the unprotected bone found in the corresponding protected bone.

The 20 minute animal data indicates high uptake of the radioactivity in bone with very little activity remaining in any soft tissue. Examination of Table 3 reveals that the tibias behind the tourniquets concentrated less of the radioactive bone-seeking agents than the corresponding tibias regardless of the length of time the tourniquet remained in place or the specific complex used. However, leaving the tourniquets in place for 20 minutes provided much better protection than shorter time periods. Examples 3, 4, and 5 all showed only 20 percent to 25 percent of the unprotected radioactivity concentration in the protected tibias while Example 6 (Sm-EDTMP) was less effective at 36 percent. The tourniquet was placed in the middle of the left femur. Thus the femur was only partially protected from the systemically administered radioactive bone-seeking agents. Table 3 shows that Examples 3 and 5 (Ho-DOTMP and Sm-DOTMP) were still able to keep the concentration of radioactivity in the 20 percent to 25 percent range in the femur. Examples 4 and 6 (Ho-EDTMP and Sm-EDTMP) were only able to lower the concentration of radioactivity to 40 percent to 50 percent of unprotected concentration in the femur.

Table 1 Tibial Percentage Dose per Gram for Examples 3 to 6

	5 minute	10 minute	20 minute
Ho-DOTMP Right Tibia	7.06	7.52	6.39
Ho-DOTMP Left Tibia	3.19	3.41	1.35
Ho-EDTMP Right Tibia	1.60	6.95	5.33
Ho-EDTMP Left Tibia	1.35	3.49	1.25
Sm-DOTMP Right Tibia	7.91	6.79	8.34
Sm-DOTMP Left Tibia	4.82	3.38	2.06
Sm-EDTMP Right Tibia	6.73	6.17	5.73
Sm-EDTMP Left Tibia	5.33	2.56	2.04

Table 2 Femoral Percentage Dose per Gram for Examples 3 to 6

	20 minute
Ho-DOTMP Right Femur	5.59
Ho-DOTMP Left Femur	1.33
Ho-EDTMP Right Femur	4.31
Ho-EDTMP Left Femur	2.33
Sm-DOTMP Right Femur	6.46
Sm-DOTMP Left Femur	1.40
Sm-EDTMP Right Femur	5.44
Sm-EDTMP Left Femur	2.05

Table 3 Dose per Gram in Left Leg as a Percentage of Dose per Gram in Right Leg for Examples 3 to 6

	5 minute	10 minute	20 minute
Ho-DOTMP Tibia	45	45	21
Ho-EDTMP Tibia	84	50	23
Sm-DOTMP Tibia	61	50	25
Sm-EDTMP Tibia	79	41	36
Ho-DOTMP Femur			24
Ho-EDTMP Femur			54
Sm-DOTMP Femur			22
Sm-EDTMP Femur			38

Various modifications and variations of the described compositions and methods of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with particular preferred embodiments, it should be understood that the inventions claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art and in fields related thereto are intended to be within the scope of the following claims.

What is claimed is:

1. A method for the treatment of osteomyelitis comprising:

intramuscularly administering a composition comprising a radionuclide to a subject suffering from osteomyelitis under conditions such that said osteomyelitis is reduced.

2. The method of Claim 1, wherein said composition further comprises a ligand.

3. The method of Claim 2, wherein said ligand is a macrocyclic aminophosphonic acid.

4. The method of Claim 3, wherein said ligand is 1,4,7,10-tetraazacyclododecanetetramethylenephosphonic acid.

5. The method of Claim 2, wherein said ligand is acyclic.

6. The method of Claim 5, wherein said ligand is selected from the group consisting of ethylenediaminetetramethylenephosphonicacid, diethylenetriaminepentamethylenephosphonic acid, hydroxyethylethylenediaminetrimethylenephosphonic acid, nitrilo-trimethylenephosphonic acid, tris(2-aminoethyl)aminehexamethylenephosphonic acid, methylene diphosphonate, hydroxymethylenediphosphonate, hydroxyethylidene diphosphonate, and ethane-1-hydroxy-1,1-diphosphonic acid.

7. The method of Claim 6, wherein said ligand is ethylenediaminetetramethylenephosphonic acid.

8. The method of Claim 1, wherein said radionuclide is selected from the group consisting of Arsenic-77, Molybdenum-99, Rhodium-105, Lutetium-177, Cadmium-115, Antimony-122, Promethium-149, Osmium-193, Gold-198, Thorium-200, Samarium-153, Yttrium-90, Gadolinium-159, Rhenium-186, Rhenium-188, Holmium-166, Tin-117, Indium(In)-115, Dysprosium(Dy)-165, Lanthanum(La)-140, Ytterbium(Yb)-175, Scandium (Sc) 47, Actinium-225, Bismuth-212, and Bismuth-213.

9. The method of Claim 8, wherein said radionuclide is Holmium-166, Samarium-153, Yttrium-90 or Lutetium-177.

10. The method of Claim 1, wherein said osteomyelitis affects a bone located in a limb.

11. The method of Claim 10 further comprising the step of:

applying an arterial obstruction device to the limb or limbs affected by osteomyelitis under conditions such that arterial blood flow to said limb or limbs is obstructed before administering the composition such that some portion of the bone marrow of said subject is protected from ablation.

12. A method for the treatment of osteomyelitis comprising:

locally administering a composition comprising a radionuclide to a subject suffering from osteomyelitis at a particular site of infection under conditions such that said osteomyelitis is reduced.

13. The method of Claim 12, wherein said composition further comprises a ligand.

14. The method of Claim 13, wherein said ligand is a macrocyclic aminophosphonic acid.

15. The method of Claim 14, wherein said ligand is 1,4,7,10-tetraazacyclododecanetetramethylenephosphonic acid.

16. The method of Claim 13, wherein said ligand is acyclic.

17. The method of Claim 16, wherein said ligand is selected from the group consisting of ethylenediaminetetramethylenephosphonicacid, diethylenetriaminepentamethylenephosphonic acid, hydroxyethylethylenediaminetrimethylenephosphonic acid, nitrilo-trimethylenephosphonic acid, tris(2-aminoethyl)aminehexamethylenephosphonic acid, methylene diphosphonate, hydroxymethylenediphosphonate, hydroxyethylidene diphosphonate, and ethane-1-hydroxy-1,1-diphosphonic acid.

18. The method of Claim 17, wherein said ligand is ethylenediaminetetramethylenephosphonic acid.

19. The method of Claim 12, wherein said radionuclide is selected from the group consisting of Arsenic-77, Molybdenum-99, Rhodium-105, Lutetium-177, Cadmium-115, Antimony-122, Promethium-149, Osmium-193, Gold-198, Thorium-200, Samarium-153, Yttrium-90, Gadolinium-159, Rhenium-186, Rhenium-188, Holmium-166, Tin-117, Indium(In)-115, Dysprosium(Dy)-165, Lanthanum(La)-140, Ytterbium(Yb)-175, Scandium (Sc) 47, Actinium-225, Bismuth-212, and Bismuth-213.

20. The method of Claim 19, wherein said radionuclide is Holmium-166, Samarium-153, Yttrium-90, or Lutetium-177.

21. The method of Claim 12, wherein said particular site of infection is a bone located in a limb.

22. The method of Claim 21 further comprising the step of:

applying an arterial obstruction device to the limb or limbs affected by osteomyelitis under conditions such that arterial blood flow to said limb or limbs is obstructed before administering the composition such that some portion of the bone marrow of said subject is protected from ablation.

23. A method for the treatment of osteomyelitis comprising:

a) applying a tourniquet to a subject suffering from osteomyelitis; and

b) administering a composition comprising a radionuclide to the subject under conditions such that the osteomyelitis is reduced.

24. The method of Claim 23 wherein the radionuclide is selected from the group consisting of Arsenic-77, Molybdenum-99, Rhodium-105, Lutetium-177, Cadmium-115, Antimony-122, Promethium-149, Osmium-193, Gold-198, Thorium-200, Samarium-153, Yttrium-90, Gadolinium-159, Rhenium-186, Rhenium-188, Holmium-166, Tin-117, Indium(In)-115, Dysprosium(Dy)-165, Lanthanum(La)-140, Ytterbium(Yb)-175, Scandium (Sc) 47, Actinium-225, Bismuth-212, and Bismuth-213.

25. The method of Claim 23 wherein the composition further comprises a ligand selected from the group consisting of ethylenediaminetetramethylenephosphonicacid, diethylenetriaminepentamethylenephosphonic acid, hydroxyethylethylenediaminetrimethylenephosphonic acid, nitrilo-trimethylenephosphonic acid, tris(2-aminoethyl)aminehexamethylenephosphonic acid, methylene diphosphonate, hydroxymethylenediphosphonate, hydroxyethylidene diphosphonate, and ethane-1-hydroxy-1,1-diphosphonic acid.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/39692

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K51/06 A61P19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 02 062398 A (NEORX CORP ;FRITZBERG ALAN R (US)) 15 August 2002 (2002-08-15) page 30, line 22 page 13, line 17 -page 15, line 27; claim 1	1-3, 8-10, 12-14, 19-22
X	US 6 214 812 B1 (DIXON H B F ET AL) 10 April 2001 (2001-04-10)	1-3,8, 10-14, 19,21-24
Y	column 2, line 47-65	1-25
X	US 2001/041689 A1 (DIXON H B F ET AL) 15 November 2001 (2001-11-15)	1-3,8, 10-14, 19,21-24
Y	paragraphs '0008!,'0009!	1-25

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2003	04/04/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cattell, James

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/39692

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 059 412 A (SIMON JAIME ET AL) 22 October 1991 (1991-10-22) cited in the application column 2, line 43 -column 3, line 47	1-25
Y	US 5 066 478 A (SIMON JAIME ET AL) 19 November 1991 (1991-11-19) cited in the application column 3, line 57 -column 4, line 63	1-25
Y	US 4 897 254 A (SIMON JAIME ET AL) 30 January 1990 (1990-01-30) cited in the application column 3, line 56 -column 4, line 14	1-25
X	ODDZIALU ET AL: "An initial assessment of therapeutic value of strontium 85 isotope in treatment of chronic osteomyelitis in adults. " CHIR NARZ RUCHU ORTPO. , vol. LVIII, no. 2, 1993, pages 5454-58, XP002235556 Poland see Summary	1,12,22, 23

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 02/39692

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-25
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Although claims 1-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Although claims 1-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Although claims 1-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 1-25

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 02/39692

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 02062398	A	15-08-2002	WO	02062398 A2	15-08-2002
US 6214812	B1	10-04-2001	US	2001041689 A1	15-11-2001
			US	2002142994 A1	03-10-2002
US 2001041689	A1	15-11-2001	US	6214812 B1	10-04-2001
			US	2002142994 A1	03-10-2002
US 5059412	A	22-10-1991	AT	112689 T	15-10-1994
			AU	4700989 A	21-06-1990
			AU	639899 B2	12-08-1993
			AU	4828290 A	10-07-1990
			AU	657641 B2	16-03-1995
			AU	5068593 A	24-02-1994
			BR	8907255 A	12-03-1991
			CA	2005880 A1	19-06-1990
			CN	1046739 A ,B	07-11-1990
			CY	1902 A	15-12-1989
			DE	68918852 D1	17-11-1994
			DE	68918852 T2	11-05-1995
			DK	195990 A	16-08-1990
			EP	0375376 A2	27-06-1990
			EP	0408701 A1	23-01-1991
			ES	2061010 T3	01-12-1994
			FI	101857 B1	15-09-1998
			HK	146795 A	22-09-1995
			HU	54897 A2	29-04-1991
			IE	66391 B1	27-12-1995
			IE	940833 L	19-06-1990
			IL	92784 A	26-08-1994
			JP	2515929 B2	10-07-1996
			JP	3502936 T	04-07-1991
			KR	178966 B1	20-03-1999
			MX	18786 A	01-12-1993
			NO	903632 A ,B,	17-10-1990
			NO	964191 A	17-10-1990
			NZ	231818 A	26-05-1992
			PT	92619 A ,B	29-06-1990
			WO	9006776 A1	28-06-1990
			ZA	8909734 A	28-08-1991
			AU	563671 B2	16-07-1987
			AU	4122985 A	12-12-1985
			CA	1243603 A1	25-10-1988
			DE	3583257 D1	25-07-1991
			DE	164843 T1	07-12-1989
			EP	0164843 A2	18-12-1985
			HK	146895 A	22-09-1995
			IL	74902 A	23-12-1990
			JP	1931074 C	12-05-1995
			JP	6055681 B	27-07-1994
			JP	61022029 A	30-01-1986
			JP	1761381 C	20-05-1993
			JP	4048799 B	07-08-1992
			JP	62132892 A	16-06-1987
			NZ	211808 A	06-01-1989
			US	4898724 A	06-02-1990
			US	5064633 A	12-11-1991
			US	5066478 A	19-11-1991

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 02/39692

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5066478 A	19-11-1991	US 4898724 A	06-02-1990
		US 5300279 A	05-04-1994
		MX 9203064 A1	01-07-1992
		US 5064633 A	12-11-1991
		AU 563671 B2	16-07-1987
		AU 4122985 A	12-12-1985
		CA 1243603 A1	25-10-1988
		DE 3583257 D1	25-07-1991
		DE 164843 T1	07-12-1989
		EP 0164843 A2	18-12-1985
		HK 146895 A	22-09-1995
		IL 74902 A	23-12-1990
		JP 1931074 C	12-05-1995
		JP 6055681 B	27-07-1994
		JP 61022029 A	30-01-1986
		JP 1761381 C	20-05-1993
		JP 4048799 B	07-08-1992
		JP 62132892 A	16-06-1987
		NZ 211808 A	06-01-1989
		US 5059412 A	22-10-1991
		ZA 8502799 A	30-12-1986
US 4897254 A	30-01-1990	AU 584918 B2	08-06-1989
		AU 4743485 A	24-04-1986
		BR 8504625 A	15-07-1986
		CA 1270845 A1	26-06-1990
		DE 3577550 D1	13-06-1990
		EP 0176288 A1	02-04-1986
		IL 76329 A	31-10-1989
		JP 1935782 C	26-05-1995
		JP 6062440 B	17-08-1994
		JP 61112016 A	30-05-1986
		NZ 213567 A	27-07-1989
		ZA 8506921 A	27-05-1987